

Immunocontraception in companion animals

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Abstract

There is real need worldwide to control the population growth of companion animals. Throughout the world and particularly in the United States, overpopulation of unwanted dogs and cats is a concern for many reasons. Feral populations pose risk to native species by spread of disease and predation. That unwanted animals are humanely eradicated is of concern to many persons. The need to control population growth has led to various approaches to contraception, including immunocontraception. Concerns regarding efficacy, duration of action, harm to the individual, and species specificity are among the issues being addressed. As new technologies emerge, ethical, political, and safety issues evoke differing opinions. It is hoped that in the near future, different strategies will be developed to solve this disturbing problem.

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1. Introduction

Overpopulation of dogs and cats in the United States and in certain areas around the world is a troubling issue for many sectors of society [1]. The number of excess dogs and cats euthanatized in the United States each year is estimated to be in the millions. It is estimated that there are as many feral cats as pet cats [2]. Euthanasia, as a means of controlling the overpopulation of dog and cats is costly in many respects; financial to the authorities that must oversee these activities, emotional to those who must actually perform the euthanasia and dispose of the carcasses, and societal to all who care about this disturbing problem.

For many years, alternatives to euthanasia have been sought to deal with overpopulation of companion

animals. Immunocontraception is the process using the body's own immune system to block fertility; this is the same concept utilized by standard vaccines against bacterial, viral, and toxic agents. Many different reproductive tissues and hormones have been investigated with varying results [3]. In addition to finding the most useful antigen for a vaccine, appropriate delivery systems have proven to be a challenge in feral populations. If baiting systems are used, then species specificity becomes an issue. There is real concern regarding impact on nontarget species when one considers the implications of disseminating a contraceptive vaccine into the environment.

In spite of the many challenges, investigators are making progress in the area of immunocontraception. With scientific advances using recombinant technologies, new strategies are being developed to safely address the challenge of population control. As acceptance of these technologies occurs on a political and societal level, we can expect advances in immunocontraception.

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2. Target antigens

When developing a contraceptive vaccine, the first step is to decide which antigen will be used to stimulate the immune system. Early studies centered on the zona pellucida (ZP) which envelopes the oocyte of mammals both pre- and postovulation [4]. Crude preparations of porcine ZP were first used in a variety of species with variable, but encouraging results. As technology progressed, the ZP was found to include three subunits. These individual subunits have been investigated separately and in combination to elucidate their roles in fertilization and types of immune responses [5].

Sperm antigens were the next major group of tissue antigens studied for possible use in contraceptive vaccines. It was noted that in some infertile human couples, antisperm antibodies could be isolated in the woman. Furthermore, antisperm antibodies were induced in men after vasectomy; these antibodies reduced fertility in individuals that had their vasectomy reversed [6,7]. Therefore, sperm antigens have been used as target antigens in contraceptive vaccines [2].

The next group of target antigens was endogenous reproductive hormones. Hormonal control of reproduction was effective due to negative feedback loops involving the hypothalamus, pituitary, and gonads. The most promising hormonal antigen for immunocontraception was gonadotropin releasing hormone (GnRH) from the hypothalamus [8,9]. By blocking the action of GnRH, the entire reproductive system was inhibited. Studies now are being reported regarding the success of this antigen in companion animals [8–10]. Due to its small size (10 amino acids) GnRH is not immunogenic; thus it must be coupled with a larger protein molecule to induce an immune response [8].

Polynucleotide vaccines, considered the Third Revolution in Vaccinology, open up new possibilities in immunocontraception [11]; long-lived immunity can be induced with very small amounts of antigen. Although subunit vaccines previously were limited by not inducing a cellular response, the use of plasmids in vaccines to introduce DNA induced both humoral and cellular immunity [12,13]. Another advantage of using plasmids was the ability to induce immunity without the use of adjuvants, thereby eliminating injection-site reactions and adjuvant residues [11]. As progress is made in epitope mapping, individual epitopes of the sperm and egg proteins can be identified and used to target the immune response to those epitopes that can block fertility. Furthermore, vaccine technology is also taking advantage of progress being made in gene therapy [3].

3. Efficacy and side effects of a vaccine

In order for a vaccine to be acceptable, it first must be efficacious in a very large percentage of the vaccinated population [3]. Goals of an immunocontraceptive vaccine may differ according to the species and particular situation. In that regard, the criteria for efficacy may be quite different for the client-owned pet animal versus feral populations. For control of overpopulation in stray dogs and feral cats, vaccines should ideally induce long-lasting or permanent infertility in a very high percentage of the vaccinated animals, while concurrently blocking reproductive behavior.

When a vaccine is tested, the production of circulating neutralizing antibodies is the most frequently used measure of success of a response to the vaccine. In a contraceptive vaccine, the desired effect is to prevent pregnancy by either blocking fertilization or early embryonic development. The antibodies must get to site of concern, which in the female means the reproductive tract and its secretions. Circulating versus mucosal antibodies may have vastly different efficacies [3].

Another concern when developing a contraceptive vaccine is whether there will be undesired side effects [14]. Cytotoxic cells may attack the tissues within the ovary or testicle, thus causing destruction of the gonad itself [3]. This may or may not be a desired side effect. Another real concern is starting an autoimmune cascade in which nonreproductive tissues are attacked and destroyed, causing disease processes unrelated to contraception.

4. Delivery systems

It has always been problematic to vaccinate free-ranging species. In the larger species, darting systems have been used to administer vaccines or other pharmaceuticals [15]. However, in smaller species, darting is neither practical nor useful. Trapping is used to gain access to animals, such as the “Trap, Neuter, and Release” programs utilized in controlling feral cat populations [16]. Once trapped, these animals get quite wary and avoid traps in the future; this makes these feral animals extremely difficult to trap a second time, virtually eliminating the possibility of booster injections of an immunocontraceptive vaccine.

Baiting systems have proven to be a useful technique to administer vaccines to feral and wild populations of animals [17]. Widespread control of rabies has been successfully accomplished by distribution of a biscuit containing a recombinant viral vector that induces

protection against the rabies virus. That the reproductive tract is a mucosal effector site linked to the common mucosal system [3] suggests that the oral route could be useful in administering contraception vaccines.

If access to the animal can be accomplished, traditional injections can be used to administer a variety of vaccines. New and novel approaches have been developed that allow the use of polynucleotide (DNA) vaccines [11]. The magnitude of response and the type of response can be varied, depending on whether the vaccine is administered IM or intradermal (ID) [18]. Gene guns have been devised to administer very small amounts of a DNA vaccine directly to the dendritic cells in the skin, resulting in very efficient presentation of antigen and stimulation of immunity [19].

5. Species specificity and recombinant organisms

Evolving vaccine technologies allow the use of recombinant organisms to present antigens to the immune system. Examples of such organisms are bacterial and viral agents that either do not cause disease or have had their pathogenic properties eliminated. Vaccine strains of these organisms can be patented and approved for use in commercial vaccines. Historically, the most successful example of a live organism used as a vaccine is the vaccinia virus that allowed eradication of small pox [20]. Recombinant technology has been used to insert rabies antigens into the vaccinia virus. Successful large-scale vaccination of foxes for rabies has been accomplished by lacing edible biscuits with the recombinant vaccinia virus [21]. Another recent commercial success using a recombinant organism as a vaccine is the use of canary pox in the equine vaccine for West Nile virus (Recombitek[®], Merial, Ltd., Duluth, GA, USA). Both examples show that recombinant technology can be a powerful tool in the creation of new vaccines.

A concern for medical ethicists is the introduction of recombinant organisms into the environment; that concern is greater when the recombinant organism contains inserted contraceptive antigens. In order for a live recombinant contraceptive vaccine to be safely released into the environment, there must be a high degree of specificity for the target species [3]. Unfortunately, there is a high degree of homology among species in zona pellucida and sperm antigens, making species-specificity a very difficult task [4]. Even with proof of species specificity, the approval process of a contraceptive vaccine released into the environment would prove to be very difficult. Political realities and social concerns must be overcome before this type of

approach can be used in controlling populations of animals [3].

6. Summary

Immuncontraception holds great promise for the future control of overpopulation of dogs and cats, but many hurdles remain before such vaccines are available. Vaccines that have efficacy in only the targeted species must be produced for the vaccine to have any chance of being used in the environment. Our ability to produce a contraceptive vaccine that is efficacious in only one species is not possible at the present time [3]. Progress is encouraging in the area of vaccines against GnRH [8,9]. For animals that can be directly vaccinated, this antigen seems to hold the most promise for a vaccine that can be available in the near future. In addition to species specificity and efficacy issues, vaccinating a free-roaming population presents a unique challenge. Any vaccine used in feral or stray populations of cats and dogs must induce long-lasting immunity in a very high percentage of the vaccinated individuals, eliminating the need for frequent boosters. The cost of production must also be low enough to make the vaccine affordable. The use of recombinant organisms in contraceptive vaccines must overcome great political and societal resistance. A high degree of safety would be needed regarding the widespread distribution into the environment of a recombinant organism containing contraceptive antigens. The relatively slow emergence of contraceptive vaccines for dogs and cats reflects the complexity of the science involved in contraceptive vaccine development [3].

References

- [1] Kuehn BM, Kahler SC. The cat debate. *J Am Vet Med Assoc* 2005;224:169–73.
- [2] 2001–2002 National pet owner survey Greenwich: American Pet Products Manufacturing Association; 2002. p. 64.
- [3] Bradley MP, Eade J, Penhale J, Bird P. Vaccines for fertility regulation of wild and domestic species. *J Biotechnol* 1999; 73:91–101.
- [4] Skinner SM, Prasad SV, Ndolo TM, Dunbar BS. Zona pellucida antigens: targets for contraceptive vaccines. *Am J Reprod Immunol* 1996;35:163–74.
- [5] Hasegawa A, Hamada Y, Shigeta M, Koyama K. Contraceptive potential of synthetic peptides of zona pellucida protein (ZPA). *J Reprod Immunol* 2002;53:91–8.
- [6] Isojima S, Li TS, Ashitaka Y. Immunologic analysis of sperm-immobilising factor found in serum of women with unexplained sterility. *Am J Obstet Gynaecol* 1968;101:677–83.
- [7] Menge AC. Clinical immunologic infertility: diagnostic measures. Incidence of antisperm antibodies fertility and mechanisms. In: Dhindsa DS, Schumacher GFB, editors. *Immu-*

- nological aspects of infertility and fertility regulation. Amsterdam: North Holland; 1988. p. 205–24.
- [8] Levy JK, Miller LA, Crawford PC, et al. GnRH immunocontraception of male cats. *Theriogenology* 2004;62:1116–30.
 - [9] Robbins SC, Jelinski MD, Stotish RL. Assessment of the immunological and biological efficacy of two different doses of a recombinant GnRH vaccine in domestic male and female cats (*Felis catus*). *J Reprod Immunol* 2004;64:104–19.
 - [10] Jung M, Moon Y, Cho I, et al. Induction of castration by immunization of male dogs with recombinant gonadotropin-releasing hormone (GnRH)-canine distemper virus (CDV) T helper cell epitope p35. *J Vet Sci* 2005;6(1):21–4.
 - [11] Babuik LA, Lewis J, Suradhat S, et al. Polynucleotides vaccines: potential for inducing immunity in animals. *J Biotechnol* 1999;73:1311–40.
 - [12] Wolff JA, Ludtke JJ, Ascadi G, et al. Long-term persistence of plasmid DNA and foreign gene expression in mouse muscle. *Hum Mol Gen* 1992;1:363–9.
 - [13] Davis HL, Brazolot-Millan CL, Watkins SC. Immune-mediated destruction of transfected muscle fibers after direct gene transfer with antigen-expressing plasmid DNA. *Gene Ther* 1997;4:181–8.
 - [14] Delves PJ, Roitt IM. Vaccines for the control of reproduction—status in mammals, and aspects of comparative interest. *Progress Fish Vaccinol* 2005;121:265–73.
 - [15] Kirkpatrick JF, Turner Jr JW, Liu IKM, Fayrer-Hosken R. Applications of pig zona pellucida immunocontraception to wildlife fertility control. In: *Zona pellucida glycoproteins and immunocontraception*. *J Reprod Fertil Suppl* 1996;50:183–89.
 - [16] Centonze LA, Levy JK. Characteristics of free-roaming cats and their caretakers. *J Am Vet Med Assoc* 2002;220:1627–33.
 - [17] Artois M, Mason E, Barrat J, Aubert M. Efficacy of three oral rabies vaccine baits in the red fox: a comparison. *Vet Microbiol* 1993;38:167–72.
 - [18] Feltquate DM, Heaney S, Webster RG, et al. Different T helper cell types and antibody isotypes generated by saline and gene gun DNA immunization. *J Immunol* 1997;158:2278–84.
 - [19] Raz E, Carson DA, Parker SE, et al. Intradermal gene immunization: the possible role of DNA uptake in the induction of cellular immunity to viruses. *Proc Natl Acad Sci USA* 1994;91:9519–23.
 - [20] Smith GL. Vaccine delivery systems: genetically engineered viruses as candidate vaccines. *Vaccine* 1996;14:681–3.
 - [21] Wolf KN, Elvinger F, Pilcicki JL. Infrared triggered photography and tracking plates to monitor oral rabies vaccine contact by raccoons in culverts. *Wildlife Soc Bull* 2003;31:387–91.